CLAIMS

1. A method for the expression of a recombinant protein of interest, said method comprising:

   a) culturing a host cell which expresses:

      i) one or more genes encoding the recombinant protein(s) of interest;

      ii) at least two genes encoding proteins selected from the group consisting of the chaperone proteins GroEL, GroES, DnaK, DnaJ, GrpE, ClpB and their homologs (for example, Hsp104, Ydj1 and Ssa1 in yeast); under conditions suitable for protein expression; and

   b) separating said recombinant protein of interest from the host cell culture.

2. A method according to claim 1, wherein the genes selected in step a) ii) include DnaK, DnaJ and GrpE or homologs thereof.

3. A method according to claim 2, wherein the genes selected in step a) ii) additionally include ClpB or a homolog thereof.

4. A method according to any one of claims 1-3, wherein the genes selected in step a) ii) include GroES and GroEL or homologs thereof.

5. A method according to claim 4, wherein the genes selected in step a) ii) include the DnaK, DnaJ, GrpE, ClpB, GroES and GroEL genes or homologs thereof.

6. A method for the expression of a recombinant protein of interest, said method comprising:

   a) culturing under conditions suitable for protein expression a host cell which expresses:

      i) one or more genes encoding one or more recombinant protein(s) of interest;

      ii) one or more genes encoding proteins selected from the group consisting of the chaperone proteins GroEL, GroES, DnaK, DnaJ, GrpE, ClpB and their homologs (for example, Hsp104, Ydj1 and Ssa1 in yeast);

iii) one or more genes encoding proteins selected from the group consisting of the small heatshock proteins of the IbpA family and/or the IbpB family and/or their homologs; and

b) separating said recombinant protein of interest from the host cell culture.

7. A method according to any one of the preceding claims wherein the levels of the respective chaperone proteins are controlled.

8. A method according to claim 7, wherein said levels of chaperone proteins are controlled by expressing the genes encoding the respective chaperone proteins from different promoters.

9. A method according to claim 7 or claim 8, wherein the respective chaperone proteins are expressed using expression systems of different strength.

10. A method according to any one of claims 7-9, wherein said chaperone proteins are over-expressed relative to the expression levels that occur naturally in non-recombinant cells.

11. A method according to any one of the preceding claims, wherein the levels of the chaperone proteins relative to the recombinant protein(s) of interest are controlled by expressing the genes encoding the respective proteins from different promoters or by using different polymerases.

12. A method according to any one of the preceding claims, wherein in culturing step a) of the method, a block in protein synthesis is imposed, for example, by the addition of an effective amount of a protein synthesis inhibitor to the culture system, once a desired level of recombinant protein of interest has accumulated.

13. A method according to claim 12, wherein the chosen protein synthesis inhibitor is chloramphenicol, tetracycline, gentamycin or streptomycin.

14. A method according to any one of claims 1-13, wherein in culturing step a) of the method, a reduction in gene transcription is imposed, for example, by removal of any agents that are effective to induce recombinant protein expression (such as IPTG for Lac repressor controlled genes), or via the addition of a transcription blocking compound (such as glucose for catabolite repressable genes), once a desired level of recombinant protein of interest has accumulated

15. A method for the expression of a recombinant protein of interest, said method comprising:

a) culturing a host cell which expresses:

i) one or more genes encoding the recombinant protein(s) of interest;

ii) one or more genes encoding one or more proteins selected from the group consisting of the chaperone proteins GroEL, GroES, DnaK, DnaJ, GrpE, ClpB and their homologs (for example, Hsp104, Ydj1 and Ssa1 in yeast); under conditions suitable for protein expression;

b) imposing a block in protein synthesis, for example, by the addition of an effective amount of a protein synthesis inhibitor to the culture system, once a desired level of recombinant protein of interest has accumulated; and

c) separating said recombinant protein of interest from the host cell culture.

16. A method for the expression of a recombinant protein of interest, said method comprising:

a) culturing a host cell which expresses:

i) one or more genes encoding the recombinant protein(s) of interest;

ii) one or more genes encoding one or more proteins selected from the group consisting of the chaperone proteins GroEL, GroES, DnaK, DnaJ, GrpE, ClpB and their homologs (for example, Hsp104, Ydj1 and Ssa1 in yeast); under conditions suitable for protein expression;

b) imposing a reduction in gene transcription, for example, by removal of any agents that are effective to induce recombinant protein expression (such as IPTG for Lac repressor controlled genes), or via the addition of a transcription blocking compound (such as glucose for catabolite repressable genes), once a desired level of recombinant protein of interest has accumulated; and

c) separating said recombinant protein of interest from the host cell culture.

17. A method according to claim 15 or claim 16, wherein said host cells additionally expresses one or more genes encoding proteins selected from the group consisting of the small heatshock proteins of the IbpA family and/or the IbpB family and/or their homologs.

18. A method according to any one of claims 14 to 17, wherein in step a) ii), a combination of chaperone proteins is expressed as recited in any one of claims 2-6.

19. A method according to any one of claims 15, claim 17 or claim 18, wherein the chosen protein synthesis inhibitor is chloramphenicol, tetracycline, gentamycin or streptomycin.

20. A method according to any one of the preceding claims, wherein said cultured host cell is a prokaryotic cell, such as an *E. coli* cell, a *Lactococcus* cell, a *Lactobacillus* cell or a *Bacillus subtilis* cell, or a eukaryotic cell such as a yeast cell, for example a *Pichia* or *Saccharomyces* yeast cell, or an insect cell, for example after baculoviral infection.

21. A method according to any one of the preceding claims, wherein an optimised yield of said recombinant protein of interest is manifested by increasing the level of *de novo* protein folding.

22. A method according to any one of claims 1-20, wherein an optimised yield of said recombinant protein of interest is manifested by increasing the level of *in vivo* refolding of aggregated, or misfolded soluble, recombinant protein.

23. A method according to any one of claims 1-20, wherein an optimised yield of said recombinant protein of interest is manifested by increasing the level of *in vitro* refolding of aggregated, or misfolded soluble, recombinant protein.

24. A method according to claim 20, wherein an optimised yield of said recombinant protein is manifested by increasing the level of *de novo* protein folding in combination with an increased level of *in vivo* protein refolding and/or *in vitro* protein refolding.

25. A method according to any one of claims 21-24, wherein said increased level of folding or re-folding results in increased solubility of the recombinant protein of interest.

26. A method according to any one of claims 21-25, wherein said increased level of folding or re-folding results in increased activity of the recombinant protein of interest.

27. A method for increasing the degree of refolding of a recombinant protein of interest, said method comprising adding a composition containing a chaperone protein to a preparation of the recombinant protein of interest *in vitro*.

28. A method according to claim 27, wherein a combination of chaperone proteins as recited in any one of claims 2-6 is added to the preparation of the recombinant protein of interest.

29. A method according to claim 27 or claim 28, wherein the preparation of the recombinant protein of interest is a preparation of soluble recombinant protein that has been precipitated *in vivo*.

30. A method according to claim 27 or claim 28, wherein the preparation of the soluble recombinant protein of interest is a preparation of *in vitro* precipitated recombinant protein.

31. A method according to any one of claims 27-30, wherein said composition containing the chaperone protein(s) is added after removal of any agents that are effective to induce soluble recombinant protein expression (such as IPTG for Lac repressor controlled genes) or after addition of a transcription blocking compound (such as glucose for catabolite repressable genes).

32. A method according to any one of claims 27-31, additionally comprising the step of imposing a block in protein synthesis, such as by the addition of an effective amount of a protein synthesis inhibitor to the culture system.

33. A method according to claim 32, wherein the chosen protein synthesis inhibitor is chloramphenicol, tetracycline, gentamycin or streptomycin.

34. A method according to any one of the preceding claims, wherein the refolding temperature and time course of refolding are controlled.

35. A method according to any one of claims 27-34, additionally comprising the use of one or more proteins selected from the group consisting of the small heatshock proteins of the IbpA family and/or the IbpB family and/or their homologs

36. The use of one or more genes encoding one or more proteins selected from the group consisting of the chaperone proteins GroEL, GroES, DnaK, DnaJ, GrpE, ClpB and their homologs (for example, Hsp104, Ydj1 and Ssa1 in yeast), and one or more genes encoding proteins selected from the group consisting of the small heatshock proteins of

the IbpA family and/or the IbpB family and/or their homologs, in the manufacture of a medicament for the treatment of disease in which the presence of aggregated proteins are implicated.

37. The use of one or more selected from the group consisting of the chaperone proteins GroEL, GroES, DnaK, DnaJ, GrpE, ClpB and their homologs (for example, Hsp104, Ydj1 and Ssa1 in yeast), and one or more genes encoding proteins selected from the group consisting of the small heatshock proteins of the IbpA family and/or the IbpB family and/or their homologs, in the manufacture of a medicament for the treatment of disease in which the presence of aggregated proteins are implicated.

38. A method of treating a patient suffering from a disease in which the presence of aggregated proteins is implicated, comprising administering one or more genes encoding one or more proteins selected from the group consisting of the chaperone proteins GroEL, GroES, DnaK, DnaJ, GrpE, ClpB and their homologs (for example, Hsp104, Ydj1 and Ssa1 in yeast), and one or more genes encoding proteins selected from the group consisting of the small heatshock proteins of the IbpA family and/or the IbpB family and/or their homologs.

39. A method of treating a patient suffering from a disease in which the presence of aggregated proteins is implicated, comprising administering one or more proteins selected from the group consisting of the chaperone proteins GroEL, GroES, DnaK, DnaJ, GrpE, ClpB and their homologs (for example, Hsp104, Ydj1 and Ssa1 in yeast), and one or more proteins selected from the group consisting of the small heatshock proteins of the IbpA family and/or the IbpB family and/or their homologs.

40. The method of claim 38 or claim 39, wherein the disease is late or early onset Alzheimer's disease, SAA amyloidosis, hereditary Icelandic syndrome, multiple myeloma, or a spongiform encephalopathy.